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# A NEW SPECIES OF CRENOTHRIX (C. MANGANIFERA)

BY D. D. JACKSON

WITH ONE PLATE

There is no bacterial growth, barring those which actually produce disease, which has excited more interest or caused more trouble than the "Giant Bacterium" known as *Crenothrix*. Its chemical and biological aspects present so many interesting peculiarities and its connection with filtered or ground water-supplies is so important that the organism is certainly worthy of special study.

At the present writing but one species of the genus *Crenothrix* has been described. In reality there are three species which fall under this head. The mode of occurrence, conditions for growth, and life history of these three species are similar in almost every respect. The measurements and minor differences are shown later in tabulated form, but the most noticeable distinguishing feature between the three species is the difference in the chemical which it precipitates from the water.

The first and well-known species precipitates hydroxid of iron and occurs in reddish brown flocs or strings. This is the genus described as *Hypheothrix* by Kützing previous to 1850 and is probably the same as that found in 1852 by Kühn in a drain pipe leading from the reservoir of a garden in Bunzlau (Silesia). Shortly after the latter discovery, Rabenhorst in his Algae of Saxony and Central Europe called the organism *Leptothrix kühniana*. In a subsequent book, however, Rabenhorst[1] described the same organism as *Hypheothrix kühniana*. In 1870 Cohn[2] gave a description of this organism and called it the Brunnenfaden or well-thread, and proposed the technical name of *Crenothrix*. He found the organism widely distributed in well waters, and as he observed but one species, and that with a peculiar and prolific spore formation, he gave it the specific name of *polyspora*. Since that time the species has gone under the name of *Crenothrix polyspora* or *Crenothrix kühniana*.[3]

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[1] Flora Europaea Algarum Aquae Dulcis, etc., Bd. II, p. 88. Leipzig, 1865.

[2] "Über den Brunnenfaden (*Crenothrix polyspora*)," etc. *Beiträge zur Biologie*, Bd. I. pp. 117-31.

This is the organism which in 1878 caused the serious contamination of the Tegel water-supply of Berlin,[4] which resulted in the abandonment of the wells sunk near Tegel Lake as a source of supply. The same organism in 1887 caused serious trouble in the water from the sand filters at Rotterdam,[5] which derived their supply from the river Maas. This set of filters was also abandoned. During the last ten years *Crenothrix kühniana* has given great trouble in numerous cities, both abroad and in this country, and much expense has been involved in the replacing or treatment of the supplies affected.

The species which precipitates iron is the one which had always been the cause of trouble. Of recent years two other species have been brought into play in the degenerating of ground waters. One of these has been known as *Leptothrix ochracea* and has not previously been described as seriously affecting water-supplies. It will be shown that this organism should properly come under the genus *Crenothrix* and that the material precipitated, although yellowish in color, consists chiefly of alumina.

The other species has never been described and is rarer even than the second. This organism precipitates manganese, and, inasmuch as large quantities of manganese are rarely found in water-supplies, it has had less opportunity to develop in noticeable amounts. The author has found it in but two places and in only one in large numbers. The three species may be easily distinguished when in bulk from the color of the precipitates produced.

The iron bacterium is from yellowish red to reddish brown in color, depending upon the age of the growth. The aluminum bacterium is from pure white to yellowish white. The manganese bacterium is from brown to almost black. The characteristics of this latter species would entitle it to the name of *Crenothrix*

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[3] W. T. Sedgwick, "On *Crenothrix kühniana*." *Tech. Quar.*, Vol. VIII, No. 4. Nov., 1890.

[4] O Brefeld and W. Zopf, Bericht an den Magistrat der Stadt Berlin über die von ihnen angeführten Untersuchungen des Tegeler Wassers. Berlin, 1879.
W. Zopf, Entwickelungsgeschichtliche Untersuchung der *Crenothrix polyspora*, Die Ursache der Berliner Wassercalamität. Berlin, 1879.

[5] Hugo De Vries, "Die Pflanzen und Thiere in den dunkeln Raumen der Rotterdamer Wasserleitung." Bericht über die Biologischen Untersuchungen der Crenothrix-Commission zu Rotterdam vom Jahre 1887, pp. 1-55. Jena, 1890.

*manganifera,* and as the species producing alumina and formerly called *Leptothrix ochracea* belongs under the genus *Crenothrix,* as will be shown later, it should properly be called *Crenothrix ochracea,* while the old and well-known species which precipitates iron should be called *Crenothrix kühniana,* as *kühniana* is the specific name which takes precedence in point of time.

All three species occur chiefly in ground waters and only grow with rapidity when the dissolved oxygen is lacking, or nearly so, and when the special salts are present which they precipitate. The presence of much organic matter seems to favor the growth, but the two former conditions are absolutely necessary. The absence of light and the presence of carbonic acid in the water are also usual conditions and seem to favor growth.

Wherever these organisms have been the cause of serious trouble, the growth has taken place in driven well tubes and has stopped up the tubes, or has been carried on to clog the well screens, or it has occurred in the bottom or sides of filter galleries and dug wells and in the dead ends of water pipes. Except in the latter case, the original cause is improper conditions for filtration, or too heavy a draught upon the water, thus using up the dissolved oxygen and causing conditions which would favor the reduction and solution of iron, manganese, or aluminum from the soil. These salts occur in the water as sulfate or in combination with carbonic acid and organic matter, and are incorporated into the sheath of the organism. The salts seem to be oxidized by the growth and are then precipitated with it. This precipitate not only clogs up the pipes and screens, but makes the water very disagreeable in appearance and unfit for domestic use or for industrial purposes.

A brief description of the genus will be given, and then a table showing the differences between the three species will be presented.

GENUS *Crenothrix.* Filaments cylindrical, transversely divided into cells of very unequal length. Surrounded by a gelatinous envelope which is usually filled with a flocculent precipitate, yellow, red, brown, or black. Filaments unbranched, but the bacteria within have a faculty of leaving their outgrown sheath often at the side by means of a splitting in the cell wall.

> Bacteria in sheath 1.5 μ wide and of very unequal length. Forms spores, 1 to 6 μ in diameter. Habitat, stagnant or improperly filtered water.

The manner of multiplication of the genus is described by Sedgwick[6] in his "Data of Filtration" as follows:

"The first method of multiplication depends upon the capacity of all the cells composing a filament, if they become isolated in any way, to develop each a new filament. When, for instance, the cells free themselves from their old rust sheath, and before they form a new envelope are broken apart by the current or by a blow, obviously we have supplied the germ for one or several flocs. But besides this, the plant possesses other methods of multiplication. In the thicker filaments the cells divide not merely transversely, as in the thinner threads, but also lengthwise. Thereupon each cell divides into two or four adjoining cells. These latter separate and form the so-called microspores. In a filament in which this process has occurred, as soon as the cells have left their sheath in the usual manner, these microspores escape as innumerable, entirely distinct masses, which speedily become scattered in the water. Each microspore, immediately after its escape, begins to divide. Division takes place in all directions, and a little cluster soon arises composed of hundreds of cells. The sheath with which each cell envelopes itself changes in this case into a "slime" or jelly, which, however, becomes colored in the usual manner with the oxide of iron. By means of this "slime" the whole mass is stuck or pasted to any part of the walls of the reservoir or to any objects within it. After some time the course of development changes, and each cell of the cluster grows into a filament. In some of these the cells divide transversely only and form the so-called macrospores; in others the above described microspores arise."

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[6] *Tech. Quar.*, Vol. III, No. 4. Nov., 1890.

If we take into consideration the foregoing descriptions it will be seen from the following table that the three organisms belong to the same genus but represent quite sufficient differences to rank them as separate species.

TABLE I

| | CRENOTHRIX KÜHNIANA | CRENOTHRIX MANGANIFERA | CRENOTHRIX OCHRACEA |
|---|---|---|---|
| Color of precipitate in sheath | Reddish brown...... | Dark brown or black | Very light buff (color of ochre) |
| Filaments. | Nearly straight...... | Nearly straight...... | Curved, often spiral |
| | 1.5–5 μ thick, widening at end to 6–9 μ.<br>6–15 μ long | 8–15 μ thick ........<br>35–125 μ long....... | 1.5–3 μ thick<br>60–150 μ long |
| | Articulated and in places interrupted | Articulated and in places interrupted | Not articulated but interrupted |
| | Transverse division distinct | Transverse division distinct | Transverse division indistinct |
| Bacteria in filament. | 1.5×2 μ ............ | 1.5×3–6 μ .......... | 1.5–2 μ×4–9 μ |
| | Distinct when treated with HCl | Distinct when treated with HCl | Not distinct when treated with HCl |
| | Easily stained with Loeffler solution | Easily stained with Loeffler solution | Difficultly stained with Loeffler sol. |
| | Slightly rounded ends | Decidedly rounded ends | Slightly rounded ends |
| Macro-spores... | 1–6 μ, spherical diameter | 3×4 μ, oval......... | 1.5×2 μ, oval |
| Micro-spores... | 1 μ ................. | 1–1.5 μ ............. | 1 μ |

A chemical analysis of the precipitates produced by the various species shows that about one-third of the entire constitution of the organism consists of the special oxide which it precipitates, another third or more consists of organic matter, and the remainder, which may be merely accidental and not a true constituent, is largely silica and aluminum silicate.

The following table shows these interesting facts:

TABLE II

ANALYSIS OF PRECIPITATES IN WATER CAUSED BY THE THREE SPECIES OF CRENOTHRIX

| | CRENOTHRIX KÜHNIANA READING, MASS. | CRENOTHRIX MANGANIFERA NEWTON, MASS. | CRENOTHRIX OCHRACEA BROOKLYN, N.Y. |
|---|---|---|---|
| Oxid of iron. $Fe_2O_3$ | **31.6** % | 14.4 % | 14.7 % |
| Oxid of manganese, $Mn_2O_3$ | 0.0 | **33.9** | 0.0 |
| Oxid of aluminum, $Al_2O_3$ | 5.7 | 1.5 | **33.3** |
| Silica, $SiO_2$ | 11.1 | 12.5 | 5.9 |
| Aluminum silicate | 10.4 | 8.0 | 7.6 |
| Undetermined mineral matter | 4.2 | 1.8 | 2.3 |
| Total mineral matter | 63.0 % | 72.1 % | 63.8 % |
| Organic matter | 37.0 | 27.9 | 36.2 |
| Total min. and organic matter | 100.0 % | 100.0 % | 100.0 % |

It is of interest to show that the waters which produce the various species contain salts which especially favor the growth of that particular species. The following table gives the analyses of waters which have produced large quantities of the species named:

TABLE III

ANALYSIS OF WATER IN WHICH THE THREE SPECIES OF CRENOTHRIX GROW

| PTS. PER MIL. | CRENOTHRIX KÜHNIANA READING, MASS. | CRENOTHRIX MANGANIFERA NEWTON, MASS. | CRENOTHRIX OCHRACEA BROOKLYN, N. Y. |
|---|---|---|---|
| $Fe_2O_3$ | **3.1** | 0.2 | 0.3 |
| $Mn_2O_3$ | 0.0 | **2.0** | 0.0 |
| $Al_2O_3$ | 0.6 | 0.5 | **2.0** |
| $SiO_2$ | 22.6 | 10.4 | 22.9 |
| $CaCO_3$<br>$MgCO_3$ | 24.0 | 12.0 | 98.0 |
| $CaSO_4$<br>$MgSO_4$ | 8.0 | 16.0 | 1.0 |
| NaCl | 5.3 | 7.8 | 7.5 |
| Undetermined | 3.5 | 3.3 | 2.2 |
| Total mineral | 67.1 | 52.2 | 133.9 |
| Total organic | 22.4 | 5.8 | 21.1 |
| Total mineral and organic | 89.5 | 58.0 | 155.0 |

It will be seen that waters which produce *Crenothrix kühniana* contain a predominating amount of iron, that waters which produce *Crenothrix manganifera* contain a predominating amount of manganese, and that waters which produce *Crenothrix ochracea* contain a predominating amount of aluminum. It is probable also that this aluminum must be present in the form of sulfate or in combination with carbonic acid and organic matter. Sulfate of aluminum in quantity has been found in waters near these wells and may occur as such in the waters affected.

*Crenothrix kühniana* was grown artificially in an atmosphere of air and carbonic acid. The cultures were in a mixture of agar and gelatine containing ferrous and manganous sulfates, and were kept in the dark at room temperature. The growth was not very luxuriant, but took place in sufficient amount to show that the organism in growing threw down but little manganese, but selected the iron to precipitate. This was also proved in stagnant solutions containing salts of manganese and iron.

In the same way cultures of *Crenothrix manganifera* were grown in solutions containing iron and manganese, and although the growths were but slight they showed the dark color of the manganese predominating in the precipitate.

It is highly probable that *Crenothrix ochracea* would in the same way always select aluminum in predominating amount to precipitate, as out of many hundreds of waters containing *Crenothrix* which have been examined there has been no case where this species has thrown down a predominating amount of iron or manganese, and in no case has *Crenothrix kühniana* been known to throw down a predominating amount of aluminum. In other words, the species always precipitates in predominating amount the special hydroxid ascribed to it.

*Crenothrix kühniana* was also grown on gelatin agar in an atmosphere of hydrogen. The growth was normal in measurement and developed well-marked filaments; but although the medium contained ferrous sulfate, none of the iron was thrown down. This is because there was no opportunity for oxidation.

We can conclude that *Crenothrix kühniana* is very common, because iron in quantity is a common constituent of water; that the species *C. ochracea* is rather uncommon because aluminum sulfate or aluminum combined with carbonic acid is less common in water; that the species *C. manganifera* is very rare because manganese seldom occurs in sufficient quantity in water to produce a noticeable growth of the organism; that the growth of all three species is due to lack of oxygen with a consequent reducing action in the soil or water; that this reducing action may be produced by stagnation in the bottoms of ponds or by improper or too rapid filtration in wells or filter galleries.

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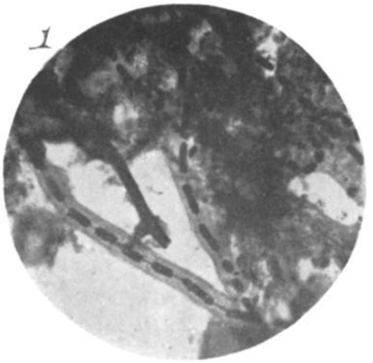
## PLATE X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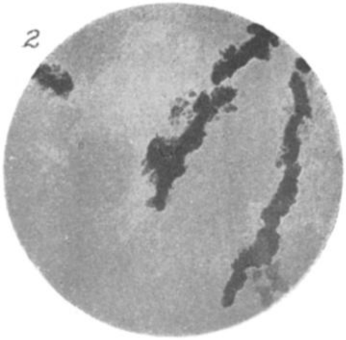
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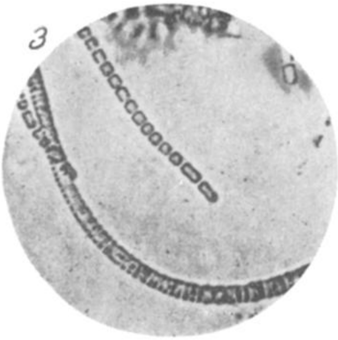
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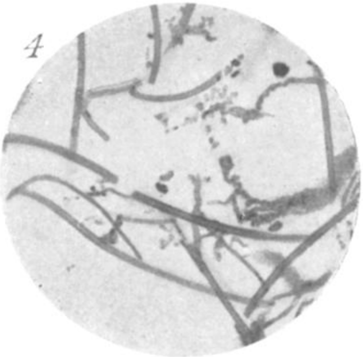
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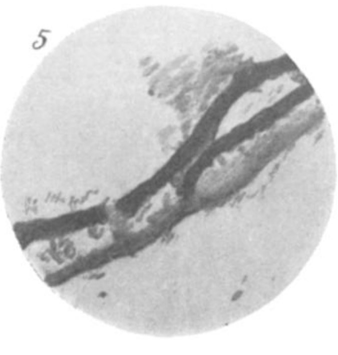
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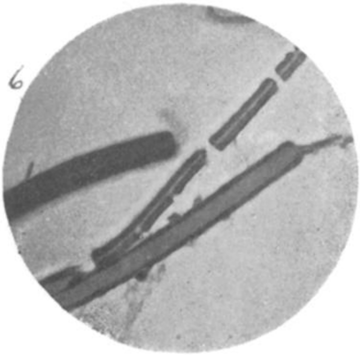
6

## EXPLANATION OF PLATE

### Plate X

Fig. 1. *Chrenothrix manganifera.*

Magnification 800 diameters.

The manganese has been removed from the gelatinous sheath in the lower half of the field by means of acid and the bacteria stained with Loeffler solution in order that the filaments and the inclosed bacteria may be clearly shown. Near the lower edge of the field may be seen a case of multiplication by transverse division.

The thread-like attachment connecting each bacterium with the next has probably never before been photographed and can not be shown except by photography. This photograph and Nos. 4 and 6 were taken from the author's preparations by Dr. R. B. Fitz-Randolph of the Hoagland Laboratory, Brooklyn, N. Y.

Fig. 2. *Crenothrix kühniana.*

Magnification 250 diameters.

In this photograph the filaments and bacteria are entirely covered by the precipitated iron in the gelatinous sheath. Nos. 2 and 5 are from photographs by G. C. Whipple.

Fig. 3. *Crenothrix kühniana.*

Magnification 1,200 diameters.

A young growing filament is shown in the upper part of the field, while in the lower part is a filament filled with microspores. Photograph by George W. Rafter.

Fig. 4. *Crenothrix ochracea.*

Magnification 800 diameters.

The filaments may be seen through the translucent precipitated alumina in the sheath.

Fig. 5. *Crenothrix kühniana.*

Magnification 800 diameters.

The iron has been nearly all dissolved by acid and the filmaments with their articulations may be seen.

Fig. 6. *Crenothrix manganifera.*

Magnification 800 diameters.

The manganese has been dissolved by acid and the articulated filaments are distinctly shown.

Note the variation in size and general appearance in the three species represented by Nos. 4, 5, and 6.